IJFSNPHT

ISSN: 0975 – 8712
IJFSNPHT (2015), 7(3):13-29

Safety Assessment of Heavy Metals in *RACHURUS TRACHURUS* Frozen Fish Species imported and sold in ZARIA METROPOLIS, NIGERIA

A. Abubakar[1], A. Uzairu[2], P. A. Ekwumemgbo[3], O. J. Okunola[4]
1-3 Department of chemistry, Ahmadu Bello University, Zaria Kaduna State, NIGERIA
4 Department of Applied chemistry, Federal University Dutsin-Ma, P.M.B. 5001 Dutsin-Ma, Katsina State, NIGERIA
Aabubakar88@ymail.com, adamuuzairu@gmail.com

ABSTRACT
This study aimed at the assessment of safety levels of heavy metals; cadmium, lead, mercury, iron and nickel (Cd, Pb, Hg, Fe and Ni) in frozen *Trachurus Trachurus* fish species imported and sold in Zaria metropolis. The fish species were assessed on the bases of their import batches and variations of heavy metals localization in various tissues/organs (skin, muscles, gills, liver, intestine, kidneys, brain and bones). Accumulation levels of the metals concentration in various tissues were determined with flame AAS via microwave digestion. The trend in the metals accumulation were in this other Fe > Hg > Pb > Ni > Cd. There is significant variations ($p<0.05$) in the heavy metals accumulation across the batches of various tissues; majorly liver, intestine and kidneys accumulates highly than the corresponding skin, muscles, gills, brain and bones. Also, the concentrations of heavy metals obtained across the entire tissues of *Trachurus Trachurus* fish species was found to be higher rather than showed significance ($p<0.05$) as compared to the safety limits recommended by FAO/WHO. However, human health with respects to consumptions of fish livers, intestine and kidneys may face considerable risk from ingestion of toxic metals at unacceptable concentrations.
Key words: Frozen fish, *Trachurus Trachurus*, Heavy metals, Risk assessment, Safety.

INTRODUCTION

Fish constituted an important part of the international trade that remains vital in its export and import among many countries, accounting for about ten percent of the total agricultural exports in value terms [1]. In Nigeria, fish were regarded as the most valuable source of animal protein and on rapid increase in consumption demand than any other source [2 - 4]. The major regard for fish as source of animal protein, was due to its availability and often less expensive than beef [5 - 8]. Fish provides not only high-value protein, but also a wide range of essential micronutrients, including various vitamins (D, A and B), minerals (including calcium, iodine, zinc, iron and selenium) and polyunsaturated Omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid) [1]. With few exceptions for selected species, fish offers advantage of low saturated fats, carbohydrates and cholesterol [1].

In contrast to the potential health benefits of dietary fish intake, its high demand have leads to the establishment of several preservation methods such as frozen, drying, smoking and canning method among others [1, 7]. Frozen method (of fish preservation) gives rise to distribution of different fish species across the Nation for people to have the taste of their own choice. Although, lodgment of materials into water bodies from natural and anthropogenic activities such as agricultural practice, industrial/mining activities and domestic sewages erosion/weathering, oceanic surge etc., may render high concentration of pollutants, usually industrial effluents, PCBs, dioxins, pesticides, heavy metals etc. Heavy metals have received considerable attention due to their toxicity and bioaccumulation in different organs of aquatic animals and fish [8 - 13]. Fish supplies from many origins are mostly in frozen form, this only preserved it from decomposition by slowing down some biochemical activities, but do not have any impact on the presence of heavy metals contaminants [14]. However, the presence of metals such as cadmium, lead and mercury above tolerance level in fish, may pose potential health implication. Hence, these heavy metals due not have any nutritional value. According to Trites *et al*. [13], fish occupy the apex of the food chain and ones became affected with heavy metals substances, the entire food chain would also be affected.

In Nigeria, there are limited reports on safety assessment of heavy metals in imported frozen fish (*Trachurus Trachurus* species). Several studies proclaimed that, wild captured marine fish accumulates heavy metals to a greater extend that can be of health concern [15 - 19]. Comparative assessment of fish on heavy metals accumulation reveals that, *Trachurus Trachurus* fish species showed high tissues accumulation of metals among others [20]. Studies of marine life on heavy metals contamination also indicate that, fish represents the best bio-indicator among others [20 - 23]. Thus, there is need for continuous assessment of heavy metals safety in fish supplies especially *Trachurus Trachurus* species that are supplied from different origins. This is important because fish trade generally have influenced the dietary patterns of sea foods globally. Therefore, this study was aimed at the assessment of safety levels of heavy metals accumulation in *Trachurus Trachurus* fish species imported and sold in Zaria, Nigeria. This is achieved through the following objectives: (1) the variation in the heavy metals accumulation in various fish tissues, (2) the variation in the production batches and (3) the variation impact of heavy metals pollutant between two major fishing zones (areas).

EXPERIMENTAL

Quality Assurance

The reagents used in this work were grades of chemicals from Sigma-Aldrich Company. Double distilled and de-ionized water were used throughout the experimentation except where indicated otherwise. Dissecting surgical blades, plastic containers and trays were washed and rinsed with distilled water. Glass wares were soaked in 10% HNO_3 (for 24hours) and rinsed with distilled water. Preparations of all the standard solutions were performed in a clean laboratory environment. All the samples were digested along with the blanks. Quantification of metallic

content of the digested samples and the blanks was carried out with the aid of Varian AA240 Fast Sequential Flame Atomic Absorption Spectrophotometer (AAS) in MULTI-USER SCIENCE RESEARCH LABORATORY (MSRL), Ahmadu Bello University Zaria.

To ensure that the Varian AA240 Fast Sequential Atomic Absorption Spectrometer remained calibrated at the course of the experimentation, the standards were analyzed after every ten runs. In the absence of reference standard materials, the nitrate salts of the metals were used to prepare multi-element standard solution (MESS) for spiking recoveries in the validation of digestion method. The analyzed samples were spiked and run in AAS again and the concentrations of the metal contents were determined from the calibration curve. The amounts of spiked metals recovered were used to calculate the percentage recoveries. Determinations were carried out in triplicates per sample of fish tissues. Dilution factors of the collected data were corrected by calculations and the values were presented in the units of mg/kg.

Fish sampling

Twelve (12) different batches of *Trachurus Trachurus* species were purchased from frozen fish depot at Sabon-Gari in Zaria metropolis, considering six (6) batches each obtained from two fishing origins (Russian; RS labeled 1 - 6 and Europe; EU labeled 6 – 12). The general information on the cartons leaflets include fishing areas (zones) as fish destination or origins, name/address of the producer, plant code number, country of origin, fishing zone, production date, expiry date, product name, scientific/Latin name, size, batch number, net weigh, packing line/time and storage temperature. Samples were collected in clean polythene bags, labeled and transported immediately to laboratory. In the laboratory the samples were stored in refrigerator and remained freeze prior to experiment. The incremental number of fish drawn per carton was based on the method specified by FAO [24]. Since the cartons net weight was found to be less than 50kg, thereby three (3) incremental fish were drawn randomly and composites from each batch of the carton, because some organs are tiny.

Fish pre-treatment and dissection

Frozen fish samples were thawed, washed with distilled water and then allowed to attain room temperature in desiccators before dissection. The skin and muscle were removed following *Tru-cut* method by Baker *et al*. [25]: skin ware lift from the dorsal region on the first side of the fish just below the dorsal fin using a sterilized notched needle. The outer barrel was inserted to a depth of about 1cm into the fish muscle tissue. The 2cm long notched needle (inner barrel) was then extended into the flesh. The containment cover (i.e. sharp outer barrel) slides over the extended needle to cut the tissue and capture it within the notch. The needle was then withdraw, the barrel opened and tissue slug remove with stainless steel tweezers (which were washed between samples) and placed in a labeled plastic Petri dish. While at the other side, soft scales were removed and the skin was cut-off firmly making sure no part of the muscle was attached and placed in its labeled drying dish.

The gills, brain, liver, intestine, kidney and bone was dissected based on the modified NIVA method by Rosseland *et al*. [26]. Dissections were done on plane plastic tray to separate sample organs: The operculum gill cover was lift up and cut to expose the entire gill ash, filament and the rake. The abdominal wall was cut through the tail using tweezers and laid the fish on its right side with head to left. The bile bladder was removed from the liver and then whole pooled liver was also freed onto the abdominal wall and cut out. The whole intestine was cut out and placed in to its drying container. The roof of the head was cut horizontally from the nostrils through the end of the skull in order to exposed the brain and fetch out and cut accordingly. Bones were de-fleshed and cut out entirely from the head to the tail. The organs were dried to constant weight at 80^0C on plastic Petri-dish and cooled in desiccators and powdered in porcelain mortar and pestle.

Digestion

The digestion of the sample organs were based on the Microwave-assisted wet digestion method as described by Taghipour and Aziz [27]: 1g dry weight samples organs were placed in polytetrafluoroethylene (PTFE) tube for microwave. Digestion reagents (mixtures of 6ml ultra-pure Nitric acid, 65% and 2ml hydrogen peroxide, 35% in a ratio of 3:1) were added and placed in a microwave oven. The heating took place for 2minutes and then cooled to temperature of 25^0C. The cleared solution were also diluted with de-ionized water to 50ml for skin, muscle, gills, liver, intestine, kidney, brain and liver and then filtered using Whatman filter paper ($90mm$). The levels of Cd, Pb, Hg, Fe and Ni in the samples were determined with AA240 Fast Sequential Atomic Absorption Spectrophotometer (AAS). In contrast to the original method by Taghipour and Aziz [27], modification was made based on the adjustment of heating duration in microwave oven. In their work they used 20minutes period of digestion, but in this work, attempting to digest fish tissues samples beyond 2minutes lead the sample inside polytetrafluoroethylene (PTFE) tube to burn leaving behind carbon residue. Hence, our modification of this method came in digestion duration (2mins instead of 10mins).

RISK ASSESSMENT

Risk assessment in this study was evaluated by considering only the muscles tissues, because it is the edible part of fish consumed by the population of fish consumers in Zaria metropolis, Nigeria. The evaluation includes the Heath Quotient (HQ), Daily Intake Metal (DIM) and Health Risk Index (HRI) according to methods by Sajjad *et al*. and Okunola *et al*. [28, 29].

Hazard Quotient (HQ)

The risk of human health contamination with the intake of heavy metal pollutants in fish was characterized by Hazard Quotient (HQ). It is a determined ratio to the

metal reference dose (RD) that can entails an estimate of metal hazard on the population in the latter life with fish consumption. If the HQ value is less than one (1) then the metal will pose no risk on the population due to fish consumption. However, if HQ value is greater than one (1) then the population would experience risk of hazardous metals. The equation (1) below was used.

$$HQ = \frac{[W_{fish}] \times [M_{fish}]}{R_fD \times B_o} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 1.}$$

Where, W_{fish} is the dry weight of edible fish consumed per day ($gday^{-1}$), M_{fish} is the concentration of metal in the fish ($mgkg^{-1}$), R_fD is the metal reference dose ($mgkg^{-1}d^{-1}$); $1.0x10^{-3}$, $3.5x10^{-3}$, $1.0x10^{-4}$, $7.0x10^{-1}$, and $2.0x10^{-2}mgkg^{-1}day^{-1}$ for cadmium, lead, mercury, iron and nickel respectively [30] and B_o is the average body weight (kg).

Daily Intake of Metal (DIM)

The daily intake of metals (DIM) was calculated to estimate the daily loading of metals into the body system (via the consumption of fish species specified in this study) of a specified body weight of a consumer. This would entail the relative bioavailability of the metals exposure with the consumption of edible part of *Trachururs Trachurus* fish species. The daily intake of metals (DIM) was determined by the equation below:

$$DIM = \frac{C_{metal} \times D_{fish} \times C_{factor}}{B_o} \ldots\ldots \text{Equation 2.}$$

Where, C_{metal} is the concentration of heavy metals in the fish ($mgkg^{-1}$), D_{fish} is the daily nutritional intake of fish ($gday^{-1}$), C_{factor} is the factor for conversion of fresh fish to dry constant weight. For *Trachurus Trachurus* fish Species in this study, C_{factor} was considered as 0.2821 as computed by the equations below as cited in USEPA [31].

In this study, the daily intake of fish for nutritional requirement was 100g for adults with average body weight of 70kg (age range from 18years and above), 80g intake rate for children with average body weight of 48kg (age range 6 - 18years) and 60g intake rate for children with average body weight of 19kg (age range 6years and below) recommended using the method by Portier *et al*. [32] as cited in USEPA [33].

$$C_{factor} = IR_{ww} - IR_{dw} \ldots\ldots\ldots\ldots\ldots \text{Equation 3.}$$

$$IR_{ww} = IR_{dw}\left[\frac{100-W}{100}\right] \ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 4.}$$

Where, IR_{dw} is the dry weight intake rate; IR_{ww} is the wet weight intake rate and W is the percent water content of the raw muscles (in this study was 71.79%)

Health Risk Index (HRI)

The health risk index (HRI) for the populations through the consumption of contaminated fish were assessed based on daily intake of metals (DIM) relative to reference oral dose (R_fD) for each of the studied metal. This is an index justifying individual's risk of heavy metals exposure. The HRI value of less than one (1) implies safe and considered acceptable, otherwise the fish may pose risk of heavy metals exposure. The following formula was used for the calculation of HRI.

$$HRI = \frac{DIM}{R_fD} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 5.}$$

Statistical analysis

The data were expressed as the metal concentration in the tissues across the batches of the two fishing origins (RS and EU), with the means and standard deviation. To show whether there is significant difference between the batches, Post-Hoc analysis of variance (Duncan) was used and the Pearson correlation (r) was used to establish the degree of relationship among the tissues based on the analyzed metals across the fishing origins, using a statistical software package (IBM SPSS version 20).

RESULTS AND DISCUSSION

Quality assurance

As shown in Table 2, the results of recoveries (for the spiked fish tissues) obtained for the investigated metals (Cd, Pb, Hg, Fe and Cd) varied between the ranges of 90.7% to 111.0%. Comparison of the recoveries data in this study gives that the values are within the range of 90% to 120% and these were in compliance with the Standard Operating Procedure (SOP) [34, 35]. Acceptable recoveries were obtained in all cases, which show that, the digestion method used for fish samples tissues and the AAS analyses were reliable.

Metal concentrations

The statistical results of heavy metals; cadmium, lead, mercury, iron and nickel (Cd, Pb, Hg, Fe and Ni) concentrations (mg/kg) in the tissues/organs (skin, muscle, gills, liver, intestine, kidneys, brain and bones) of *Trachurus Trachurus* across two fishing origins including the mean, standard deviation and Post-Hoc test analysis are summarized in Tables 3 to 7. Analysis of variation between fish samples collected from the two fishing origins; Russia (RS) and Europe (EU) and the sample batches within the same zone showed significant differences ($P<0.05$). The results of correlation analysis among the tissues/organs of the fishing zones across the studied metals are shown in Tables 8 to 17 (appendix).

Cadmium (Cd)

The cadmium concentrations shown for the tissues of both RS and EU fish species were presented in Table 3. The concentrations of cadmium in the tissues of RS fish species were ranged between 0.925mg/kg to 5.142mg/kg shown for the skin and livers tissues respectively. While for EU fish species the concentrations of cadmium recorded in the studied tissues were ranged between 0.367mg/kg to 6.517mg/kg shown for the muscles and livers tissues respectively. The results of statistical analysis shows that, significant differences ($P<0.05$) were recorded in some batches of RS fish species and these are shown among the batches of skin, muscle, brain and bones tissues. However, non-significance differences ($p<0.05$) recorded among the batches of RS tissues were shown; for the gills tissues were between batch 1 and 4, 2 and 5; for the liver tissues were between batch 3, 5 and 6, 4 and 6; for the intestine tissues were between batch 2 and 3 only; and for the kidneys tissues were between

batch 1 and 4 only. While for EU batches, the significance differences recorded were shown among the batches of skin, muscles, gills and bones tissues. Hence, the non-significance differences ($p<0.05$) recorded were shown; for the liver tissues were between batch 1 and 3, 2 and 4, 3 and 5; for the intestine tissues were between batch 2 and 6 only; for the kidneys tissues were between batch 3 and 6 only; and for the brain tissues were between batch 5 and 6 only. The significant differences shown among the batches of RS (skin, muscle, brain and bones) and EU (skin, muscles, gills and bones) tissues implies that, contaminations with cadmium across these batches are related to impacts of different sources. While, the non-significant differences shown among the batches of RS (gills, liver, intestine and kidneys) and EU (liver, intestine, kidneys and brain) tissues implies that, these batches were homogeneity contaminated with cadmium from similar impacts sources and hence. Looking at the both origins, the non-significant differences shown among batches involves the fish digestive tissues. The fish digestive organs are the major sites responsible for cadmium storage or detoxification.

The results of correlation analysis presented in Table 8 for cadmium concentrations in the tissues of RS fish species shows that, significant ($p<0.05$) positive correlations were recorded between; the skin versus intestine, muscles and kidney tissues; muscles verses gills, intestine and kidneys tissues; and brain versus bone tissues. Hence, significant ($p<0.05$) negative correlation shown, were between the gills versus liver tissues only. While, the results of correlation analysis for tissues of EU fish species presented in Table 9 shows that significant ($p<0.05$) positive correlations were recorded between; skin versus muscles, liver and bone tissues; muscles versus liver and bones tissues; liver and bone tissues; and kidneys versus bones tissues. Hence, significant negative correlation was not recorded in the tissues of EU fish origin. The prevailing variations in the batches of RS fish species leading to existence of both significant positive and negative correlations were as a result of differences in the source of cadmium contamination. Positive correlations shown among tissues, indicate that, contaminations with cadmium were from similar sources or the accumulation routes in to the tissues portray via similar mechanism (i.e. uptake was governed by similar physico-chemical conditions).While, negative correlations among other tissues were implies contamination with cadmium were from different sources. With regards to results statistical analysis, similarly the results of correlation analysis indicates some batches were homogeneity contaminated with cadmium from similar impacts sources. While cadmium contaminations among others batches are related to impacts of different sources. In lined with the both analysis, however, cadmium contaminations (as with both RS and EU fish origin) are related to impacts from both natural and anthropogenic activities.

Table 3 shows, the highest mean concentrations of cadmium (with regards to both RS and EU species) were shown in batches of livers tissues 5.142mg/kg and 6.517mg/kg respectively. The literature of metals accumulations in various fish tissues shows in coinciding sequence that, highest level of cadmium accumulations are mostly shown in the liver tissues. For instance, the study by Abolfazl and Maryam [36] found high level of cadmium (4.67mg/kg) concentrations was shown in the liver tissues of *Platycephalus Indicus* species, as well as Yilmaz [37] found 3.32mg/kg in liver tissues of *Mugil Cephalus* fish species. Despites the high level of cadmium found in liver tissues of the present reports, some studies shows that other fish species can give rise to cadmium accumulation more than as in the present report. This can be seen in the study by Storelli *et al*. [38] in *Sphyrma Zygaena* (19.81mg/kg) species and Simon *et al*. [39] in *A. Thalassinus* (13.35mg/kg) fish species. Also the studies by Gil *et al*. [40], Sausan *et al*. [41] on *Mytilus Galloprovincialis*, *Protothaca Thaca* fish species indicate that liver tissues among others are the major sites of cadmium bio-accumulations. The higher level of the cadmium in the liver relative to other tissues may be attributed to the high coordination of metallo-thionein protein with the cadmium [42]. In addition, the liver is the principal organ responsible for detoxification, transportation and storage of toxic substances [43]. It is an active site of pathological effects induced by contamination [44]. Also, the concentrations of cadmium in the intestine and kidneys irrespective to any batch were higher than the results obtained in the skin, gills, bones and brain tissues. This is because these tissues play roles in the process of absorption, storage and excretion of ingested feed by the fish. These entails that, cadmium are highly stored in tissues/organs that are involve in metabolism process.

In contrast, the lowest levels of cadmium mean concentrations are shown in the skin (0.925mg/kg) and muscles (0.367mg/kg) tissues of RS and EU batches respectively. These tissues (skin and muscles) are regarded as inactive tissues and as such can only store low concentrations of metals due to absence of metallo-thionein proteins. This fact was found in agreement with the studies by many authors, for instance Simon *et al*. [39] studies on *A. Thalassinus* and *J. Belangeri* species similarly showed, low cadmium levels were in the muscles (0.027mg/kg) and skin (0.035mg/kg) tissues, Copet *et al*. [45] from the eastern Mediterranean Sea, Kalay *et al*. [46] from the northeast Mediterranean Sea and Yazkan [47]. Furthermore, Table 3 mean concentrations of cadmium shows the entire tissues across the batches of both fishing zones were more pronounced and found above the recommended safety limit specified by FAO [24]. This implies that both natural and anthropogenic activities such as seasonal changes, volcanic eruptions, different oceanic surge and industrials discharge must have plays role that leads to variations in the accumulation of cadmium across the batches.

Table 1. Microwave operation

Operation	Output
Machine model number	MW028A-MG720
Power (output)	700W
Turntable Diameter	255m
Function/power operation	High (100%)
Digestion time	2minutes

Table 2. Mean percentage recoveries with the standard deviation of heavy metals from spiked fish tissues

Tissues	Heavy metal	Concentrations of the sample (mgL^{-1})	Concentrations of spiked sample (mgL^{-1})	% Recovery±SD
Skin	Cd	0.020	1.130	111.0±0.05
Muscle	Pb	0.146	1.112	96.6±0.20
Gills	Hg	1.808	2.800	99.2±0.35
Liver	Fe	5.403	6.373	97.0±0.15
Intestine	Ni	0.151	1.150	99.9±0.05
Kidneys	Cd	0.062	1.111	104.9±0.31
Brain	Pb	0.185	1.092	90.7±0.45
Bones	Hg	1.760	2.792	103.2±0.40

Average of eight observations from three replicate analyses of each of analyzed fish tissues spiked samples

Table 3. Concentrations of cadmium in the tissues of *Trachurus Trachurus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO[24]
Skin(RS)	1.000^{a}	0.950^{b}	0.950^{c}	1.050^{d}	1.100^{e}	0.500^{f}	0.925±0.025	0.100
Skin(EU)	0.300^{a}	0.450^{b}	0.350^{c}	0.350^{d}	0.450^{e}	0.500^{f}	0.400±0.015	
Muscle(RS)	1.050^{a}	1.050^{b}	1.050^{c}	1.050^{d}	1.000^{e}	0.500^{f}	0.950±0.045	
Muscle(EU)	0.250^{a}	0.400^{b}	0.350^{c}	0.350^{d}	0.350^{e}	0.500^{f}	0.367±0.020	
Gills(RS)	3.650^{a}	4.200^{b}	1.050^{c}	1.250^{a}	1.050^{b}	0.250^{d}	1.908±0.025	
Gills(EU)	0.350^{a}	0.500^{b}	0.450^{c}	0.350^{d}	0.400^{e}	0.450^{f}	0.417±0.040	
Liver(RS)	6.050^{a}	0.900^{b}	7.750^{c}	9.150^{d}	3.950^{cd}	3.050^{cd}	5.142±0.020	
Liver(EU)	2.950^{a}	3.450^{b}	6.750^{ac}	2.250^{b}	1.550^{c}	22.150^{d}	6.517±0.015	
Intestine(RS)	2.350^{a}	2.600^{b}	2.650^{b}	2.450^{c}	2.450^{d}	1.750^{e}	2.375±0.025	
Intestine(EU)	1.200^{a}	2.900^{b}	1.650^{c}	1.000^{d}	0.650^{e}	2.000^{b}	1.567±0.005	
Kidneys(RS)	3.100^{a}	1.650^{b}	1.550^{c}	3.150^{a}	1.350^{d}	0.650^{e}	1.908±0.005	
Kidneys(EU)	2.050^{a}	0.900^{b}	3.600^{c}	1.200^{d}	0.500^{e}	1.700^{c}	1.658±0.010	
Brain(RS)	1.200^{a}	1.300^{b}	1.550^{c}	1.300^{d}	0.600^{e}	1.100^{f}	1.175±0.025	
Brain(EU)	0.600^{a}	0.350^{b}	0.300^{c}	0.250^{d}	0.300^{e}	0.400^{f}	0.367±0.030	
Bones(RS)	1.300^{a}	2.000^{b}	2.050^{c}	1.500^{d}	1.200^{e}	1.700^{f}	1.625±0.005	
Bones(EU)	0.850^{a}	0.700^{b}	0.500^{c}	0.350^{d}	0.500^{e}	3.500^{e}	1.067±0.015	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 4 Concentrations of lead in the tissues of *Trachurus Trachurus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO[24]
Skin(RS)	8.300^{a}	10.500^{b}	17.150^{c}	15.000^{c}	19.950^{d}	19.250^{d}	15.025±0.025	0.400
Skin(EU)	18.550^{a}	18.700^{b}	16.550^{abc}	18.750^{cd}	16.100^{a}	19.650^{d}	18.050±0.015	
Muscle(RS)	7.300^{a}	10.350^{b}	14.600^{c}	18.600^{d}	18.550^{d}	16.600^{d}	14.333±0.010	
Muscle(EU)	15.100^{a}	14.950^{ab}	16.000^{bc}	16.000^{bd}	16.400^{c}	18.350^{d}	16.133±0.020	
Gills(RS)	7.100^{a}	12.800^{b}	14.700^{c}	17.500^{d}	18.100^{de}	16.950^{de}	14.525±0.030	
Gills(EU)	17.400^{a}	17.050^{b}	17.600^{ac}	17.150^{bc}	16.450^{ad}	18.700^{d}	17.392±0.010	
Liver(RS)	8.250^{a}	12.650^{b}	18.200^{c}	17.600^{c}	19.050^{d}	18.700^{d}	15.742±0.040	
Liver(EU)	18.100^{a}	16.050^{ab}	18.000^{c}	17.200^{ac}	17.050^{bd}	19.950^{d}	17.725±0.045	
Intestine(RS)	9.050^{a}	13.750^{b}	15.650^{c}	18.600^{d}	17.450^{de}	18.850^{e}	15.558±0.030	
Intestine(EU)	16.100^{a}	15.700^{a}	17.250^{b}	17.750^{c}	18.250^{d}	19.500^{e}	17.425±0.020	
Kidneys(RS)	7.600^{a}	13.350^{b}	17.200^{c}	18.400^{d}	18.550^{de}	18.200^{de}	15.550±0.005	
Kidneys(EU)	15.800^{a}	16.700^{b}	14.800^{ab}	14.100^{ab}	17.650^{c}	19.500^{d}	16.424±0.015	
Brain(RS)	9.250^{a}	13.300^{b}	17.350^{c}	17.150^{cd}	17.850^{de}	19.150^{e}	15.675±0.010	
Brain(EU)	16.700^{a}	17.150^{ab}	17.050^{c}	16.150^{ab}	20.950^{d}	20.350^{d}	18.058±0.025	
Bones(RS)	10.100^{a}	12.300^{b}	16.600^{c}	17.000^{d}	18.250^{de}	16.800^{de}	15.175±0.030	
Bones(EU)	16.100^{a}	15.250^{ab}	17.650^{ac}	15.650^{bc}	21.350^{d}	19.600^{d}	17.600±0.015	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 5 Concentrations of mercury in the tissues of *Trachurus Trachurus* (mgkg^{-1}) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO[24]
Skin(RS)	84.900^{a}	97.500^{b}	94.350^{c}	51.450^{a}	53.300^{bc}	54.950^{bc}	72.742±0.010	0.500
Skin(EU)	56.050^{a}	60.450^{b}	63.100^{c}	79.500^{a}	85.450^{bc}	63.450^{bc}	68.000±0.015	
Muscle(RS)	90.600^{a}	94.450^{b}	98.900^{c}	51.150^{a}	60.900^{bc}	56.650^{bc}	75.442±0.010	
Muscle(EU)	54.250^{a}	57.850^{b}	69.400^{c}	76.100^{a}	79.700^{b}	65.750^{bc}	67.175±0.020	
Gills(RS)	90.400^{a}	93.600^{b}	95.600^{c}	52.100^{a}	56.650^{b}	58.250^{c}	74.433±0.025	
Gills(EU)	60.050^{a}	66.550^{b}	67.950^{c}	82.900^{a}	83.000^{b}	65.750^{c}	71.033±0.005	
Liver(RS)	89.550^{a}	90.100^{b}	93.700^{c}	48.150^{a}	55.800^{b}	58.900^{c}	72.700±0.010	
Liver(EU)	51.500^{a}	68.250^{b}	71.750^{c}	79.950^{a}	82.950^{b}	59.600^{c}	69.000±0.025	
Intestine(RS)	90.600^{a}	89.650^{b}	96.400^{c}	51.700^{ab}	56.150^{ab}	59.550^{c}	74.008±0.010	
Intestine(EU)	63.400^{a}	63.400^{b}	71.250^{c}	83.150^{ab}	80.800^{ab}	63.000^{c}	70.833±0.030	
Kidneys(RS)	95.550^{a}	100.200^{b}	88.050^{c}	1.550^{ac}	55.500^{ab}	71.400^{bc}	68.708±0.015	
Kidneys(EU)	56.550^{a}	67.900^{b}	75.550^{c}	82.600^{ac}	69.900^{ab}	83.050^{bc}	72.592±0.010	
Brain(RS)	92.650^{a}	99.100^{b}	95.500^{ac}	60.700^{bc}	60.750^{b}	60.550^{a}	78.208±0.005	
Brain(EU)	56.200^{a}	68.450^{b}	73.500^{ac}	84.700^{bc}	64.100^{b}	72.700^{a}	69.942±0.015	
Bones(RS)	88.000^{a}	94.400^{b}	95.300^{c}	55.500^{a}	56.450^{bc}	56.200^{bc}	74.308±0.035	
Bones(EU)	59.800^{a}	66.550^{b}	72.800^{c}	83.850^{a}	69.000^{bc}	68.950^{bc}	70.158±0.020	

Values in each row marked by the same superscript letter are not significantly different at P<0.05

Table 6 Concentrations of iron in the tissues of *Trachurus Trachurus* (mgkg^{-1}) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WO[48]
Skin(RS)	36.050^{a}	20.250^{b}	12.500^{b}	7.900^{a}	68.250^{c}	43.550^{c}	31.417±0.005	0.800
Skin(EU)	57.700^{a}	66.100^{b}	53.900^{c}	54.250^{d}	443.00^{ab}	40.950^{ab}	119.32±0.10	
Muscle(RS)	26.900^{a}	2.950^{a}	3.050^{ac}	79.050^{d}	52.000^{d}	47.950^{c}	35.317±0.020	
Muscle(EU)	45.250^{a}	50.800^{b}	101.50^{ac}	183.55^{bd}	25.200^{ac}	26.200^{bd}	72.08±0-.035	
Gills(RS)	265.65^{a}	148.50^{b}	174.65^{bc}	127.15^{ac}	132.00^{ab}	298.40^{a}	191.06±0.025	
Gills(EU)	225.55^{a}	175.00^{b}	203.10^{c}	181.05^{c}	138.05^{a}	305.60^{d}	204.73±0.015	
Liver(RS)	270.15^{a}	183.80^{b}	163.30^{ac}	273.70^{d}	228.05^{ac}	261.85^{d}	230.14±0.035	
Liver(EU)	1000.5^{a}	1081.85^{b}	1102.05^{c}	910.70^{ac}	356.50^{ab}	565.95^{bc}	836.26±0.005	
Intestine(RS)	128.00^{a}	54.250^{b}	42.450^{ac}	258.85^{d}	63.400^{ce}	160.50^{de}	117.91±0.010	
Intestine(EU)	580.10^{a}	1028.15^{b}	799.85^{c}	205.00^{ac}	54.200^{ac}	96.750^{b}	460.68±0.045	
Kidneys(RS)	250.20^{a}	53.650^{b}	36.800^{c}	127.15^{d}	139.20^{e}	90.100^{ac}	116.18±0.035	
Kidneys(EU)	898.35^{a}	258.00^{b}	1156.3^{bc}	706.90^{d}	158.80^{ab}	64.750^{bc}	540.52±0.035	
Brain(RS)	142.75^{a}	66.950^{ab}	86.250^{c}	83.000^{ab}	143.20^{d}	175.90^{e}	116.34±0.005	
Brain(EU)	200.40^{a}	90.200^{b}	69.700^{ab}	92.000^{c}	110.20^{d}	74.250^{ab}	106.13±0.005	
Bones(RS)	58.800^{a}	63.750^{ab}	49.700^{ac}	79.850^{d}	156.80^{e}	134.95^{e}	90.642±0.015	
Bones(EU)	190.30^{a}	869.70^{b}	124.60^{c}	779.00^{bd}	133.25^{ad}	71.750^{a}	361.43±0.010	

Values in each row marked by the same superscript letter are not significantly different at P<0.05

Table 7. Concentrations of nickel in the tissues of *Trachurus Trachurus* (mgkg^{-1}) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO[49]
Skin(RS)	7.450^{a}	7.100^{b}	6.600^{a}	9.450^{c}	9.150^{c}	10.800^{d}	8.425±0.015	0.200
Skin(EU)	15.200^{a}	13.850^{a}	17.950^{b}	19.400^{c}	22.600^{d}	21.300^{d}	18.383±0.010	
Muscle(RS)	6.750^{a}	6.800^{ab}	7.900^{bc}	9.800^{cd}	11.000^{de}	12.250^{e}	9.083±0.010	
Muscle(EU)	13.100^{a}	14.950^{b}	17.250^{c}	19.500^{d}	21.900^{e}	20.600^{e}	17.883±0.020	
Gills(RS)	8.000^{a}	7.750^{a}	7.300^{ab}	8.900^{bc}	9.300^{cd}	11.050^{d}	8.717±0.005	
Gills(EU)	14.000^{a}	13.350^{b}	18.250^{ac}	18.250^{c}	21.800^{d}	1.300^{a}	14.492±0.025	
Liver(RS)	7.700^{a}	8.650^{b}	9.050^{c}	8.450^{bd}	10.900^{de}	12.600^{e}	9.558±0.005	
Liver(EU)	13.250^{a}	15.850^{ab}	20.200^{bc}	20.000^{c}	23.100^{d}	3.700^{a}	16.017±0.005	
Intestine(RS)	7.550^{a}	11.050^{b}	7.650^{ab}	8.100^{bc}	11.800^{cd}	13.100^{d}	9.875±0.010	
Intestine(EU)	13.600^{a}	13.850^{a}	18.250^{b}	21.650^{c}	22.300^{d}	5.700^{e}	15.892±0.005	
Kidneys(RS)	8.350^{a}	8.700^{ab}	9.250^{bc}	9.650^{cd}	12.450^{de}	13.450^{e}	10.308±0.005	
Kidneys(EU)	14.350^{a}	15.950^{ab}	18.600^{bc}	20.900^{c}	22.350^{c}	4.950^{a}	16.183±0.010	
Brain(RS)	7.200^{a}	8.600^{b}	9.500^{c}	8.800^{bd}	10.750^{de}	13.050^{e}	9.650±0.020	
Brain(EU)	14.750^{a}	15.500^{ab}	18.550^{bc}	20.450^{cd}	22.200^{d}	4.000^{a}	15.908±0.010	
Bones(RS)	6.600^{a}	7.500^{b}	9.100^{c}	8.850^{c}	10.400^{d}	12.850^{e}	9.217±0.015	
Bones(EU)	12.550^{a}	16.050^{ab}	19.000^{bc}	21.450^{cd}	21.650^{d}	5.450^{a}	16.025±0.010	

Values in each row marked by the same superscript letter are not significantly different at P<0.05

IJFSNPHT

ISSN: 0975 – 8712
IJFSNPHT (2015), 7(3):13-29

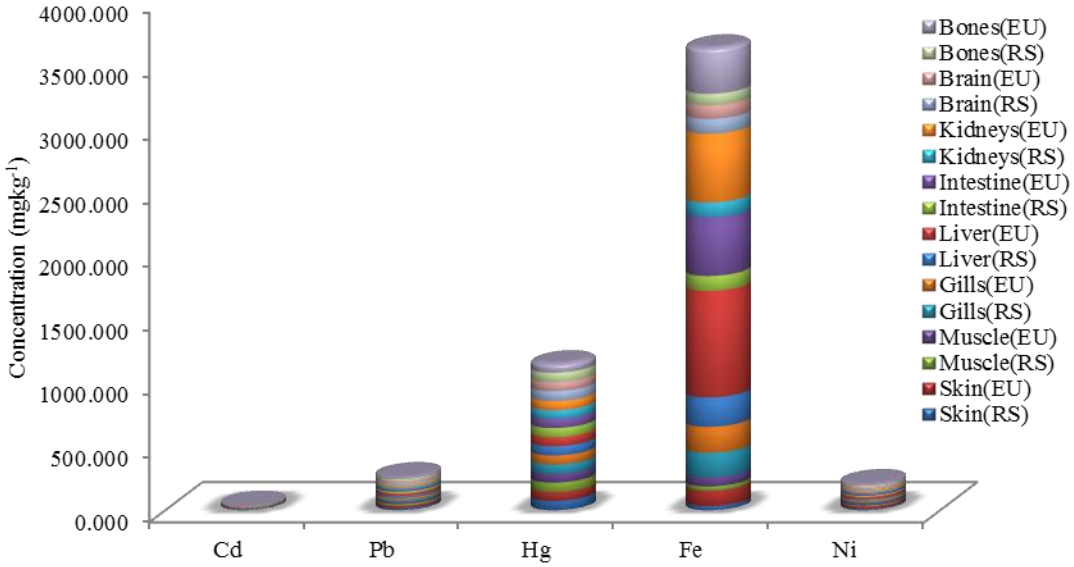

Figure1. Mean concentration of metals in the tissues of *Trachurus Trachurus* fish species

Table 18. Results of HQ, DIM and HRI for heavy metals in the muscles tissues of *Trachurus Trachurus* fish species

Heavy metal	Range (for 12 batches)	Mean±SD	Category of Individuals	HQ	DIM	HRI
Cd	0.25 – 1.05	0.659±0.33	A	0.941E+0	2.656E-4	0.266E+0
			B	1.172E+0	3.305E-4	0.331E+0
			C	2.081E+0	5.870E-4	0.587E+0
Pb	7.30–18.60	15.233±0.02	A	6.217E+0	6.139E-3	1.754E+0
			B	7.737E+0	7.638E-3	2.283E+0
			C	1.374E+1	1.360E-2	3.877E+0
Hg	51.15 - 98.90	71.309±0.02	A	1.018E+3	2.871E-2	2.874E+2
			B	1.267E+3	3.576E-2	3.576E+2
			C	2.252E+3	6.350E-2	6.353E+2
Fe	2.95 - 101.50	53.699±0.20	A	0.109E+0	2.164E-2	0.309E-1
			B	0.136E+0	2.693E-2	0.385E-1
			C	0.242E+0	4.783E-2	0.683E-2
Ni	6.75 - 21.90	13.483±0.02	A	0.963E+0	5.433E-3	0.272E+0
			B	1.198E+0	6.762E-3	0.338E+0
			C	2.129E+0	1.200E-2	0.601E+0

A = Adults (19yrs and above) B = Children (7– 18yrs) C = Children (1 – 6yrs)

Lead (Pb)

The results in Table 4 show that, the mean concentrations of lead in the tissues of RS fish species were ranged between 14.333mg/kg to 15.742mg/kg shown for the muscles and liver tissues respectively. While, mean concentrations in the tissues of EU fish species were ranged between 16.133mg/kg to 18.058mg/kg shown for the muscles and brain tissues respectively. Statistical analysis of variations shows that, significant differences ($p<0.05$) were not shown in any of the batches for both RS and EU tissues. Hence, non-significance differences ($p<0.05$) recorded for lead in the tissues of RS fish species are shown: for the skin tissues were between batch 3 and 4, 5 and 6; for the muscles tissues were between batch 4, 5 and 6; for the gills tissues were between batch 4 and 5, 5 and 6; for the liver tissues were between batch 3 and 4, 5 and 6, for the intestine tissues were between batch 4 and 5, 5 and 6; for the kidneys tissues were between batch 4 and 5, 5 and 6; for the brain tissues were between batch 4 and 5, 5 and 6; and for the bones tissues were between batch 4 and 5, 5 and 6. While, the non-significant differences ($p<0.05$) shown in the EU batches are shown: for the skin tissues were between batch 1, 3 and 5, 2 and 3, 3 and 4, 4 and 6;for the muscles tissues were between batch 1 and 2, 2, 3 and 4, 3 and 5, 5 and 6; for the gills tissues were between batch 1, 3 and 5, 2 and 4, 5 and 6; for the liver tissues were between batch 1, 2 and 4, 2 and 5, 3 and 4, 5 and 6; for the intestine tissues were between 1 and 2 only, for the kidneys tissues were between 1, 3 and 4, 2, 3 and 4; for the brain tissues were between 1, 2 and 4, 2 and 4, 5 and 6; and for the bones tissues were between 1, 2 and 3, 2 and 4, 3 and 4, 5 and 6. Recorded in both fishing zones, non-significance differences were predominantly shown across the entire batches and hence, these implied

contaminations with lead was due to impact of similar source that portrayed across the entire batches.

The correlation results for lead in the tissues of RS fish origins presented in Table 10 (appendix) shows that, significant ($p<0.01$) correlations were recorded positively across the entire tissues. While in Table 11, the correlation of lead concentrations in the tissues of EU fish origins, shows significant ($p<0.05$) positive relations were recorded also across the entire tissues. However, in both RS and EU tissues significant negative correlations were not shown. The pronounced positive correlations among tissues with regards to both RS and EU species implies that, the contamination with lead were due to impact from similar sources or the mechanisms for lead uptake in these tissues was aided by similar physico-chemical factors. Also the correlations results were in lined with the results of statistical test of significance and hence, these indicate that lead was homogeneously contaminated by similar sources across the entire batches (with respects to both fishing zones).

Table 4 shows that, lead accumulations with respect to RS fish origin were highly shown in the liver tissues (15.742mg/kg). Whereas for EU fish origins, lead accumulations were highly shown in the brain tissues (18.058mg/kg). It was reported that *Trachurus Trachurus* fish species are good indicator of lead in the aquatic environment [50]. However, the literature on several species shows that remarkable high levels of lead were shown in liver tissues, for example, Usero *et al.* [51] shows high lead concentration (5.05mg/kg) was recorded in the liver tissues of common sole frozen *Solea Vulgaris* species from the Southern Atlantic coast of Spain.

In contrast to high level of lead in liver tissues, both fish origins (RS and EU) shows lowest lead accumulations were shown in the muscles tissues. This result was in agreement with studies by many authors, who reported that muscles tissues is not an active organ and of such cannot accumulates heavy metals that can reflects the metal concentration of the surrounding habitat [11, 52, 53]. The morphological mechanism of lead transport to the fish muscles tissues was very high. This is due to the fact that, ingestion of lead and other metals take place via eating by mouth and absorption through gills or skin. The entry through the ingestion path, flows to the intestine, then absorbed, processed and distributes by the liver and exit through kidneys. These parts are referred to as target active organs where bioaccumulation of lead can takes place in the presence of mucus and metallo-thionein protein. Therefore, the higher accumulative in the liver, the lesser would be in the muscles tissues. This was also in line with the studies reported by Uzairu *et al*. and Noik *et al*. [42, 18]. Lead is classified among the most toxic heavy metals which have no known biochemical benefits to animals and humans [54]. Lead continues to pose a serious threat to the health of many children as well as adults. Lead is capable of inducing oxidative damage to brain, heart, kidneys and reproductive organs [55]. The mechanisms for lead-induced oxidative stress include the effects of lead on membranes, DNA, and antioxidant defense systems of cells [56]. Recent epidemiological and toxicological studies have reported that lead exposure causes several diseases including hypertension, kidney disease, neurodegenerative disease and cognitive impairment. *Trachurus Trachurus* species generally shows high affinity for lead accumulation in all the analyzed tissues. Hence, the fish species studied was imported from countries known to have one issue of pollution or the other a time past, the differences found in these studies might be accounted for geographic reasons. The average mean concentrations of lead in all the analyzed tissues were found to showed remarkable concentrations far above the safety limits of (0.4mg/kg) recommended by FAO [24].

Mercury (Hg)

Table 5 results shows that, mercury concentrations recorded for the tissues of RS fish species were range between 68.708mg/kg to 78.208mg/kg shown for the kidneys and brain tissues respectively. However, concentrations recorded for the tissues of EU fish origin were ranged between 67.175mg/kg to 72.592mg/kg shown for the muscles and kidneys tissues respectively. The results of statistical analysis shows that, non-significant differences ($p<0.05$) were recorded across the entire batches of both RS and EU fish species. The non-significance differences recorded with the batches of RS tissues were shown; for the skin tissues were between batch 1 and 4, 2, 4, 5 and 6, 3, 5 and 6; for the muscles tissues were between batch 1 and 4, 2, 5 and 6, 3, 5 and 6; for the gills tissues were between batch 1 and 4, 2 and 5, 3 and 6; for the liver tissues were between batch 1 and 4, 2 and 5, 3 and 6; for the intestine tissues were between batch 1, 4 and 5, 2, 4 and 5, 3 and 6; for the kidneys were tissues between batch 1, 4 and 5, 2, 5 and 6, 3, 4 and 6; for the brain tissues were between batch 1, 3 and 6, 2, 4 and 5, 3 and 4; and for the bones tissues were between batch 1 and 4, 2, 5 and 6, 3, 5 and 6. While, the non-significant differences recorded with the batches of EU tissues were shown; for the skin tissues were between batch 1 and 4, 2, 5 and 6, 3, 5 and 6; for the muscles tissues were between batch 1 and 4, 2, 5 and 6, 3 and 6; for the gills tissues were between batch 1 and 4, 2 and 5, 3 and 6; for the liver tissues were between batch 1 and 4, 2 and 5, 3 and 6; for the intestine tissues were between batch 1, 4 and 5, 2, 4 and 5, 3 and 6; for the kidneys tissues were between batch 1, 4 and 5, 2, 5 and 6, 3, 4 and 6; for the brain tissues were between batch 1, 3 and 6, 2, 4 and 5, 3 and 4; and for the bones tissues were between batch 1 and 4, 2, 5 and 6, 3, 5 and 6. The statistical results with respect to both RS and EU tissues shows that the non-significance difference recorded are shown with the entire batches and hence, these implied that the source of mercury contaminations across the batches were highly homogeneous.

The correlation results in Table 12 (appendix) for the mercury concentrations in the RS tissues shows that, significant ($p<0.01$) positive correlations were recorded across the entire tissues. While in Table 13, the

correlation results for EU tissues shows significant ($p<0.05$) positive relations were recorded across the entire tissues. However, in both RS and EU tissues, significant negative relations were not observed. Correlation results with regards to both RS and EU tissues were in lined with the results of statistical analysis, in which both analyses reported that mercury contaminations were as a result of similar sources. These justifies that the route of tissues uptake and accumulations of mercury are homogeneously contaminated across the entire batches or the uptake mechanisms were governed by similar physico-chemical factors.

Mercury mean concentrations recorded for the tissues of RS fish origins shows, higher accumulation were shown in the brain tissues (78.208mg/kg) and least were shown in the kidneys tissues (68.708mg/kg). While, for the EU tissues, kidneys exhibit higher accumulations (72.592mg/kg) and least were shown in the muscles (67.175mg/kg) tissues. Mieiro *et al.* [57] pointed out that brain is a vital organ in fish which can give rise to high accumulation of mercury. In contrast, the level of mercury in the brain tissues (of RS fish species) of this study, were higher than the level observed in the literature as in the work by Marioara *et al*. [58] that shows *Carassius Auratus Gibelio* fish species recorded highest concentration of 0.9949mg/kg and as well as in the report by Storelli *et al.* [59] that shows *Cephalopod Molluscs* fish species record the highest concentration of 0.21mg/kg. While for EU tissues, a kidney tissue shows the highest of mercury accumulations because, it is an organ of excretion, where absorption, selective re-absorption, storage and eliminations were essentially in it [60]. This fact was in compliance with the study by Meiro *et al.* [57] that shows *Liza Aurata* fish species recorded high concentration of mercury in the kidney (4.02mg/kg) tissues on low contamination ratio relationship. Mercury gets into the fish through ingestion or absorption via skin or gills from the surrounding water. Fish ingest food (contaminated with mercury), flown through gastrointestinal tract, absorbed and distributed by the liver and finally accumulates or detoxified by the kidney [61, 62]. Therefore, high mercury levels shown in the kidney tissues correspond to low detoxification process. In contrast, the lower concentrations of mercury shown in the muscles tissues of EU fish species were as a result of its role as inactive tissues. This is because the more mercury became accumulated in the digestive tissues the low its mobility would be to the inactive tissues (such as muscles, bones tissues etc.). However, the concentrations of mercury across other tissues were fairly uniform. This also shows that, contaminations with mercury were due to contributions of both natural and anthropogenic activities. Also, mean concentration of mercury with respect to entire tissues of both RS and EU fish species were above the levels reported by related studies on other fish species [63, 64] and as well as the safety limits recommended by FAO [24].

Iron (Fe)

The concentrations for iron presented in Table 6 show that, the accumulation in the tissues of RS fish origins were range between 31.417mg/kg to 230.14mg/kg shown for the skin and liver tissues respectively. While the concentrations recorded in tissues of EU fish origins, were range between 72.080mg/kg to 836.26mg/kg shown for the muscles and liver tissues respectively. The results of iron statistical analysis shows that, non-significance differences ($p<0.05$) were observed across the entire batches with respects to both RS and EU fish species. The non-significance differences ($p<0.05$) recorded for RS batches were shown; in the skin tissues between batch 1 and 4, 5 and 6; in the muscles tissues between batch 1, 2 and 3, 3 and 6, 4 and 5; in the gills tissues between batch 1, 4, 5 and 6, 2, 3 and 5, 3 and 4; in the liver tissues between batch 1, 3 and 5, 3 and 5, 3, 4 and 6;in the intestine tissue between batch 1 and 3, 3 and 5, 4 and 6, 5 and 6; in the kidneys tissues between batch 1 and 6, 3 and 6; in the brain tissues between 1, 2 and 4, 2 and 4; and in the bones tissues between batch 1, 2 and 3, 5 and 6. While, the non-significance difference ($p<0.05$) recorded for EU batches were shown: in the skin tissues between batch 1, 5 and 6, 2, 5 and 6; in the muscles tissues between batch 1, 3 and 5, 2, 4 and 6, 3 and 5; in the gills tissues between batch 1 and 5, 3 and 4; in the liver tissues between batch 1, 4 and 5, 2, 5 and 6, 3, 4 and 6;in the intestine tissues between batch 1, 4 and 5, 2 and 6, 3, 4 and 5; in the kidneys tissues between batch 1 and 5, 2, 3, 5 and 6, 3 and 6; in the brain tissues between batch 1, 3 and 6, 2, 3 and 6; and in the bones tissues between batch 1, 5 and 6, 2 and 4, 4 and 5. The results recorded that the entire batches with respect to both RS and EU tissues shows non-significant differences. This implies that, iron were homogeneous contaminated across the entire batches with respects to both fishing origins and hence, these are due to impacts of similar sources of contamination.

The results presented in Table 14 shows that, the correlations of iron concentrations in the tissues of RS fish species recorded significant ($p<0.05$) positively correlation were shown between skin versus brain and bones tissues; muscles versus liver, intestine and bones tissues; gills versus brain; liver versus intestine kidneys and bones tissues; and brain versus bones tissues. While in Tale 15, the EU tissues also shows significant ($p<0.05$) positive correlation were shown between skin versus liver, intestine and bones tissues; muscles versus kidneys; liver versus intestine and kidneys; and intestine versus bones. However, significant negative correlations were not shown in any of the RS and EU tissues. Therefore, the results of correlation analysis obtained, (with regards to both RS and EU tissues) were in lined with the results of statistical analysis, in which both analyses reported that iron contaminations were as a result of similar sources of contaminations. These justifies that the route of tissues uptake and accumulations of iron are homogeneously contaminated across the entire batches or the uptake mechanisms were governed by similar physico-chemical factors.

Iron mean concentrations were highly accumulated in the liver tissues (230.14mg/kg and 836.26mg/kg) as shown for RS and EU fish species respectively. While lower iron concentrations were shown in the muscles and skin tissues for RS and EU fish species respectively. Liver as the principal organ for storage, also stores iron to a certain higher levels in *Trachurus Trachurus* fish species. This was the attributes from high coordination of iron with metallo-thionien protein present in the liver tissues [42]. Hjeltnes *et al*. and Hulya *et al*. [65, 53] found in coinciding sequences that liver tissue accumulation iron highly than any other tissues *Salmo Salar* (246mg/kg), *Silurus Triostegus* (34.67mg/kg), *Liza Abu* (9.24mg/kg). However, significant levels of iron were shown in the intestine and gills tissues than with the skin, brain and bones tissues. The concentration of iron recorded for gills tissues would fairly predicts an estimates of the concentrations of iron present in the surrounding water body where the fish lives [53]. Whereas, the skin, brain and bones, show low concentration of iron (even though the skin tissues is in direct contact with the metals present in the water body). Hence the levels of iron are majorly contributed from the feed sources of the fish. Generally, the level of iron in all the tissues of both RS and EU fish origins were higher than the safety limits recommended by FAO/WHO [44]. Literature on iron interactions with biological tissues has reported that, excess amount of iron causes rapid increase in pulse rate and coagulation of blood in blood vessels, hypertension and drowsiness as cited in FAO/WHO [44]. Iron is essential to all forms of life and for normal human physiology processes. In humans, iron is an essential component of proteins involved in oxygen transports from the lungs to the tissues [44].

Nickel (Ni)

The results of nickel concentrations presented in Table 7 show that, the mean concentrations in the tissues of RS fish species were range between 8.425mg/kg to 10.308mg/kg shown for the skin and kidneys tissues respectively. While EU fish species shows nickel concentrations in the tissues were range between 14.492mg/kg to 18.383mg/kg shown for the gills and Skin tissues respectively. The statistical analysis of variation shows that, significant differences ($p<0.05$) were not recorded in any of the batches as with respect to both RS and EU fish origins. The non-significance difference recorded with the batches of RS tissues were shown; in the skin tissues between batch 1 and 3, 4 and 5; in the muscle tissues between batch 1 and 2, 2, 3 and 5, 3 and 4, 5 and 6; in the gills tissues between batch 1, 2 and 3, 3 and 4, 4 and 5, 5 and 6; in the liver tissues between batch 2 and 4, 4 and 5, 5and 6; in the intestine tissues between batch 1 and 3, 2, 3 and 4, 4 and 5, 5 and 6; in the kidneys tissues between batch 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6; in the brain tissues between 2 and 4, 4 and 5, 5 and 6; and in the bones tissues between 3 and 4 only. However, in the EU batches non-significance difference ($p<0.05$) were recorded in the skin tissues between batch 1 and 2, 5 and 6; in the muscles tissues between batch 5 and 6 only; in the gills tissues between batch 1, 3 and 6 only; in the liver tissue between batch 1, 2 and 6, 2 and 3; in the intestine tissues between batch 1 and 2 only, in the kidneys tissues between batch 1, 2 and 6, 2 and 3, 3, 4 and 5; in the brain tissues between batch 1, 2 and 6, 2 and 3, 3 and 4, 4 and 5; and in the bones tissues between batch 1, 2 and 6, 2 and 3, 3 and 4, 4 and 5. From the results of statistical analysis, the entire batches with respect to both RS and EU tissues show non-significant differences. Since the entire tissues show similarities of nickel uptakes across the batches, hence contaminations were as a result of similar sources. This implies that there is spontaneous leaching of nickel from the source contaminations which has impact across the entire batches.

The results of nickel correlation analysis in Table 16 (appendix) for RS tissues shows, significance ($p<0.01$) positive correlations were shown across the entire tissues. While in Table 11, EU tissues show significant ($p<0.05$) positive correlation were recorded between the skin versus muscles, gills, intestine and brain; muscle versus gills and brain; gills at $p<0.01$ versus liver, intestine, kidneys, brain and bones; liver versus intestine, kidneys, brain and bone; intestine versus kidneys, brain and bones; intestine versus kidneys, brain and bones; kidneys versus brain and bones; and also at $p<0.01$ brain versus bones. Hence, in both RS and EU tissues significant negative relations was not shown. The pronounced positive correlation in these tissues with regards to both RS and EU fish species implies that, the route for nickel contamination was strongly from similar sources or the transport mechanism of nickel uptake in these tissues was aided by similar physico-chemical factors. However, the results of correlation and statistical analysis were found in coinciding sequence and this implies that the sources of nickel contamination (with regards to both RS and EU fish) across the entire batches are homogeneous.

With regards to results presented in Table 7, nickel accumulations were highly shown in the kidneys ($10.308 mgkg^{-1}$) and skin ($18.383 mgkg^{-1}$) tissues of RS and EU fish species respectively. While, low nickel accumulations were shown in the muscles ($7.542 mgkg^{-1}$) and bones ($17.200 mgkg^{-1}$) tissues of RS and EU fish species respectively. The higher levels of nickel shown in the kidneys tissues of RS fish species are related to high coordination of nickel with the metallo-thioniene protein presents in the kidneys. Kidney is an organ next to liver that plays a vital role in digestion process. Detoxifications of trace metals by the liver tissues mostly get accumulated in the kidneys [64]. Because, detoxification process (in terms of trace metals like nickel) ineffective in the kidney tissues. This fact shows a compromise with the study by Sahar *et al*. [66] who reported that nickel analysis in *P. Indicus* and *P. Argenteus* fish species were highly accumulated in the kidney tissues. In contrast, the highest accumulations shown in the skin tissues are related to the fact that, skin is directly in contact with the metals present in the surrounding water. Thus, concentrations of heavy metals

shown in the skin tissues can also reflect the concentration of the metals in waters body where the fish lives [42]. However, the levels of nickel recorded for the liver, intestine and kidneys tissues are higher than those shown for the brain, bones and gills. Several reports show that metabolic tissues (liver, intestine and kidneys) accumulate trace metals more than any other tissues [42, 56 - 58]. This is because, these tissues act in metals storage and detoxification at the course of digestion process. In contrast, the low levels of nickel shown in the muscles and bones tissues of *Trachurus Tracurus* in this study agreed with the findings by other authors who reported that muscles and bone tissues bioaccumulation low level of trace metals (because they are inactive tissues) [59, 60, 61]. However, the concentrations of nickel shown with entire tissues (with regards to both fishing zones) were above the safety limits recommended by WHO [49].

Profile of Studied metals

The profile of the studied metals; cadmium, lead, mercury, iron and nickel (Cd, Pb, Hg, Fe and Ni) in the analyzed tissues of *Trachurus Trachurus* fish species were summarized according to the sum of their means in Figure 1. The trends in accumulation pattern were in this order: Fe > Hg > Pb > Ni > Cd. It can be seen that iron concentrations shows the highest accumulations across the entire tissues as compared to the other metals. These high levels of iron were due to contributions from both natural and anthropogenic activities which has high impacts on the water body. Natural activities such as meteor rays deposition, is an example of frequent occurrence in the Mediterranean and Russian ocean that can contributes to level of iron concentrations. Iron is an essential trace element required by all forms of life. The effects of high doses of iron in animal studies, includes symptoms of initial depression, coma, convulsion, respiratory failure and cardiac arrest [68]. Post-mortem examination reveals adverse effects on the gastrointestinal track with excess iron intake, may result in siderosis (deposition of iron in tissue); in liver, pancreas, adrenals, thyroid, pituitary and heart [69]. These are characterized by initial depression, coma, convulsion, respiratory failure and cardiac arrest [69]. Mercury is the second metal that was shown to accumulate highly in the tissues of *Trachurus Trachurus* species. Mercury, as a non-essential element, is not expected to have its uptake/elimination actively regulated and subsequently its tissue concentrations can vary in a wide range, reflecting exposure to environmental levels and feeding behavior [70]. Hence, mercury body burdens in bio-indicator species provide sensitive indications of aquatic pollution as well as the potential impact in human health [71]. However, the metal distribution within the body depends on both the fish species and the metal's properties [72]. The concentrations of lead and nickel were also highly accumulated in the fish tissues. Excess nickel exposures were associated with lung and nasal cancers [73]. Cadmium is the metal that showed least accumulation among the others. Levels of heavy metals in the tissues of *Trachurus Trachurus* fish species were studied and found highly accumulated. This implies that natural and anthropogenic human activities were essentially high in the destinations where this fish are brought. Occurrences nearby water bodies such as volcanoes, deposition of meteor rays, weathering and erosion, as well as human activities such as industrial, agricultural, domestic, mining, testing of hazardous substances from military facilities, vessels spills, chemical destruction in Mediterranean seas, oceanic surge may render high concentration of these heavy metals in the water body. Hence, in the later time these metal may accumulates in the aquatics life. In general, the levels of the investigated metals were found highly above the safety limits recommended by FAO [24] irrespective of the batch and fishing area.

Risk assessment

The risk assessment of heavy metals; cadmium, lead, mercury, iron and nickel (Cd, Pb, Hg, Fe and Ni) concentrations (mg/kg) in the muscles tissues of *Trachurus Trachurus* fish species across batches (with respect to both RS and EU fish zones); including ranges, average means, standard deviations, individuals variations, health quotient (HQ), daily intake of metal (DIM) and health risk index (HRI) are summarized in Tables 18. The individual variations was given specifications based on age differences according to following categories; category A denoted to adult of age 19years and above, category B denoted to children of age 7 – 18years and category C denoted to children of age 1 - 6years.

The mean concentration of cadmium shown in the muscles tissues of *Trachurus Trachurus* fish species across twelve (12) batches (of both RS and EU fish origins) was given as 0.659mg/kg. The assessments of individual's variation were based on the average body weight relative to ranges of age groups. The results of HQ analysis recorded a ratio values of less than one (1) for category A and values of greater than one (1) shown for category B and C respectively. These imply that, the population of adult category would not expose to high dose of cadmium with consumption of fish muscles as specified in this study. Whereas, the likely chance of cadmium exposure are shown with category A and B respectively. The DIM results for individual's daily loading of cadmium were recorded and the values were $2.656x10^{-4}$, $3.305x10^{-4}$ and $5.870x10^{-1}$ shown for individuals in category A, B and C respectively. These values correspond to HRI results in which each of the categories recorded a ratio value of less than one (1) and hence, this indicates that individuals with respects to entire categories would not expose to high dose of cadmium with the consumption of *Trachurus Trachurus* fish muscles as specified for daily intake in this study.

The mean concentration of lead shown in the muscles tissues of *Trachurus Trachurus* fish species across twelve (12) batches (of both RS and EU fish origins) was given as 15.233mg/kg. The results of HQ analysis recorded that a ratio values of greater than one (1) were

shown with each of the individual's category. These imply that, the entire population with respects to each individual's category would expose to high dose of lead metal at unacceptable concentrations with the consumption of fish as specified in this study. The DIM results show that individual's daily loading of lead were given as $6.139x10^{-3}$, $7.638x10^{-3}$ and $1.360x10^{-2}$. These correspond to HRI values of greater than one (1) shown for each of the individual's category. This indicates that, the entire categories would expose to hazard of lead metals based on consumption of *Trachurus Trachurus* fish muscles as specified in this study.

The mean concentration of mercury shown in the muscles tissues of *Trachurus Trachurus* fish species across twelve (12) batches (of both RS and EU fish origins) was given as 71.309mg/kg. The results of HQ analysis show that, the ratio values of greater than one (1) were shown with each of the individual's categories. Hence the entire population would expose to high dose of mercury at unacceptable concentrations with the consumption of fish muscles recommended for daily intake as specified in this study. The results of DIM recorded for individuals daily loading of lead were $6.139x10^{-3}$, $7.638x10^{-3}$ and $1.360x10^{-1}$. This corresponds to HRI values of greater than one (1), as shown with each of the individual categories. Since the results of HQ and HRI presented values above with ratio of greater than one (1), hence, it implies that the entire individuals consuming *Trachurus Trachurus* fish muscles of equal or above the recommended amount for daily intake specify in this study would expose to hazard of mercury.

The mean concentration of iron shown in the muscles tissue of *Trachurus Trachurus* species across twelve (12) batches (of both RS and EU fish origins) was given as 53.699mg/kg. The results of HQ analysis show that the entire categories show a ratio value of less than one (1). These imply that, the entire population would not expose to high level of iron with the consumption of fish muscles as specified in this study. The results of DIM shown for individual's daily loading of iron were given as $2.164x10^{-2}$, $2.693x10^{-2}$ and $4.783x10^{-3}$. These also correspond to HRI values of less than one (1) as shown with each of the category A, B and C respectively. However, the individual from any of the category would not expose to high level of iron with the consumption of fish muscles based on the recommended daily intake as specified in this study, otherwise there would be a benefit from iron as supplement.

The mean concentrations of nickel shown in the muscles tissues of *Trachurus Trachurus* species (across twelve (12) batches of both RS and EU fish origins) was given as 0.659mg/kg. The results of HQ analysis shows that a ratio values of greater than one (1) was for category A and while for category B and C show a value of greater than one (1) was recorded. These imply that, only adult population (first category) would not exposed to high level of nickel with the consumption of fish recommendation for daily intake as specify in this study. Whereas, the populations of both children categories B and C, would exposed to high level of nickel. The results of DIM shown for each of the individual's daily loading of nickel were given as $5.433x10^{-3}$, $6.762x10^{-3}$ and $1.200x10^{-1}$. These correspond to HRI values of less than one (1) shown for each of the category A, B and C respectively. This also indicates that individuals belonging to any of the category would not expose to high dose of nickel.

In summary, the results obtained based on the analysis of health quotient (HQ) and health risk index (HRI) as presented in Table 18, shows the population of fish consumers in Zaria metropolis would exposed to high loading dose of heavy metals; cadmium, lead and mercury at all levels with the consumption of *Trachurus Trachurus* fish species. While, the levels of trace metals iron and nickel were still within acceptable limits.

CONCLUSION

The health status of human with respect to contamination by heavy metals; cadmium, lead, mercury, iron and nickel (Cd, Pb, Hg, Fe and Ni) in the imported frozen fish *Trachurus Trachurus* species sold in Zaria metropolis, was evaluated in this study. The evaluations of heavy metals load in a large set of tissues/organs, as well as the correlation of tissue-to-tissue relations, have provided new information that can contribute to the knowledge of metal assessments. Based on the analysis obtained, the proclaim risk for human exposure to heavy metal contamination through fish consumption was significant. Since the levels of the studied heavy metals in all the analyzed tissues were above their corresponding permissible limits recommended by FAO [24], FAO/WHO [46] and WHO [47]. The population health risk from consumption of fish muscle tissues also, shows a higher chance of exposure to lead and mercury than cadmium and less with iron and nickel. However, individuals consuming fish livers, intestine and kidneys may face considerable risk from ingestion of toxic metals at unacceptable concentrations. Generally, the risk to humans cannot be excluded in relation with the consumption of imported *Trachurus Trachurus* fish from RS and EU fishing origins; it reinforced the importance to define the regulatory thresholds taking into consideration the fish consumption rate, in order to efficiently protect against hazardous heavy metals exposure. Finally, this work may provide valuable database for continuing research and assessments on the import of frozen fish in Nigeria.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the entire staff of Chemistry Department, the staff of Multi–User Science Research Laboratory and in person Aliyu Ibrahim Mu'azzamu department of pharmacology, Ahmadu Bello University Zaria for their support and analytical assistance.

REFERENCES

[1] FAO (2012). The state of world review of fisheries and aquaculture. Part 1. www.**fao**.org/docrep/016/i2727e/i2727e01.pdf

[2] Dalhatu M. and Ala A. L. (2010). Analysis of fish demand in Sokoto metropolis, Sokoto, Nigeria, Nigerian Journal of Basic and Applied Science, 18, (2): 154 - 159.

[3] Ekine D. I., Albert, C. O. and Peregba, T. A. (2012). Expenditure pattern for beef consumption in selected households in southern Nigeria. Developing Country Studies, 2, (7): 1 - 7.

[4] The World Fish Center, (2009). Reducing poverty and hunger by improving fisheries and aquaculture. Publications Catalog: Penang, Malaysia. www.worldfishcenter.org

[5] Allison E. H., Perry A. L., Badjeck M. C., Adger W. N., Brown K., Conway D., Halls A. S., Pilling G. M., Andrew N. L. and Dulvy N. K.. (2009). Vulnerability of national economies to the impacts of climate change on fisheries. Fish and Fisheries, 10: 73 - 196.

[6] Kumar B. and Mukherjee D. P. (2011). Assessment of human health risk for arsenic, copper, nickel, mercury and zinc in fish collected from tropical wetlands in India. Advances in Life Science and Technology, (2): 13 - 25.

[7] Oluwaniyi O. Omolara and Dosumu O. Omotayo. (2009). Preliminary Studies on the effect of processing methods on the quality of three commonly consumed marine fishes in Nigeria. Nigerian Society for Experimental Biology, 21, (1): 1 - 7.

[8] Ada F. B., Ekpenyong E. and Bayin, P. B. (2012). Heavy Metal Concentration in Some Fishes (Chryscihthys nigrodigitatus, Clarias gariepinus and Oreochromis nitoticus) in the Great Kiva River, Cross River State, Nigeria. Global Advanced Research Journal of Environmental Science and Toxicology, 1, (7): 184 – 189.

[9] Vinodhini R. and Narayanan M. (2008). Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). Int. J. Environ. Sci. Tech., 5, (2): 179 - 182.

[10] Babatunde A. M., Waidi O. A. and Adeolu A. A. (2012). Bioaccumulation of heavy metals in fish (hydrocynus forskahlii, hyperopisus bebe occidentalis and clarias gariepinus) organs in downstream ogun coastal water, Nigeria. Transnational Journal of Science and Technology, 2, (5): 119 – 133.

[11] Eneji S. Ishaq, Rufus S. and Annune P. A. (2011). Bioaccumulation of Heavy Metals in Fish (Tilapia Zilli andClarias Gariepinus) Organs from River Benue, North Central Nigeria. Pak. J. Anal. Environ. Chem., 12, (1): 25 - 31.

[12] Akan J. C., Abdulrahman1 F. I., Sodipo O. A. and Akandu P. I. (2009). Bioaccumulation of some heavy metals of six fresh water fishes caught from Lake Chad in Doron Buhari, Maiduguri, Borno state, Nigeria. Journal of Applied Sciences in Environmental Sanitation, 4, (2): 103 - 114.

[13] Trites A. W., Christensen V. and Pauly D. **(**2006). Effects of fisheries on ecosystems: just another top predator? In: Boyd IL, Wanless S, Camphuysen CJ (eds) Top predators in marine ecosystems. Their role in monitoring and management. Cambridge University Press, Cambridge: 11 – 27.

[14] Laila D. L. Wahidu Z. and Tajul A. Y. (2013). Effect of chilled-frozen storage on the physic-chemical, microbial and sensory quality of farmed bighead carp (Hypophthalmichthys Nobilis). Journal of fisheries and aquatic science, 2, (3): 1 – 7. http://dx.doi:10.3923/jfas.2013

[15] USEPA (2014). Provisional Peer-Reviewed Toxicity Values (PPRTV); on-line database. Accessed via http://hhpprtv.ornl.gov/ (January).Washington Department of Ecology (WDOE). Fish Consumption Rates Technical Support Document: A Review of Data and Information about Fish Consumption in Washington, Ver. 1.0 (Pub. No.11-09-050). Washington Department of Ecology, Toxics Cleanup Program. Olympia, Washington.

[16] Domingo L. Josea, Falcoa, Juan M. Llobet And Ana Bocio. (2006). Daily Intake of Arsenic, Cadmium, Mercury, and Lead by Consumption of Edible Marine Species. J. Agric. Food Chem., 54: 6106 - 6112.

[17] Mieiro C. L., Coelho J. P., Pacheco M. , Duarte A. C. and Pereira M.E. (2012). Trace elements in two marine fish species during estuarine residency: Non-essential versus essential. Mar. Pollut. Bull. http://dx.doi.org/10.1016/j.marpolbul.2012.08.033

[18] Noir Hasyimah A. K., James Noik V., Teh Y. Y., Lee C. Y. and PearlineNg H. C. (2011). Assessment of cadmium (Cd) and lead (Pb) levels in commercial marine fish organs between wet markets and supermarkets in Klang Valley, Malaysia. International Food Research Journal, 18: 795 – 802.

[19] Lillian Herger and Lorraine Edmond (2012). Assessment of mercury in fish tissue from select Lakes of Northeastern Oregon. USEPA Region 10 Report, EPA 910-R-12-001.

[20] Storelli M. M. (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). Food Chem Toxicol., 46, (8): 2782 - 8. http://dx.doi:10.1016/j.fct.2008.05.011

[21] Lasheen R. M., Fagr A., Aly A. A. and Hassan M. H. (2012). Fish as Bio Indicators in Aquatic Environmental Pollution Assessment: A Case Study in Abu-Rawash Area, Egypt. World Applied Sciences Journal, 19, (2): 265 - 275.

[22] Aftab Alam Sthanadar, Iram Alam Sthanadar, , Mudassir Shah, Pir Asmat, Muhammad Yousaf, Ali Muhammad and Muhammad Zahid (2013). White muscle as a bio-indicator of cadmium (Cd) pollution across Kalpani River Mardan, Khyber Pakhtunkhwa Pakistan. International Journal of Biosciences, 3, (10): 105 - 116. http://dx.doi.org/10.12692/ijb/3.10.105-116

[23] Tanee T., Chaveerach A, Narong C, Pimjai M., Punsombut P., Sudmoon R. (2013). Bioaccumulation of heavy metals in fish from the Chi River, Maha Sarakham Province, Thailand. International Journal of

Biosciences, 3, (8): 159 - 167. http://dx.doi.org/10.12692/ijb/3.8.159-167.

[24] FAO (2003). Heavy Metals Regulations: Part 1. Legal Notice No. 66. www.**fao**lex.**fao**.org/docs/pdf/eri42405.pdf

[25] Baker R. F., Blanchfield P. J., Paterson M. J., Flett R. J., and Wesson L. (2004). Evaluation of non-lethal methods for the analysis of mercury in fish tissue. Trans. Am. Fish Soc., (133): 568 - 576.

[26] Rosseland B. O., Massabuau J. C., Grimalt J., Hofer R., Lackner R., Raddum G., Rognerud S. and Vives I. (2001). Fish ecotoxicology, The EMERGE fish sampling manual for live fish. The EMERGE Project (European Mountain lake Ecosystems: Regionalisation, diagnostic and socio-economic valuation). http://www.mountain-lakes.org/emerge/methods/29.pdfl

[27] Taghipour V. and Aziz S. N. (2010). Determination of trace elements in canned Kilka fish marketed in Islamic Republic of Iran. World Appl. Sci. 9, (6): 704 - 707.

[28] Sajjad Khan, Robina Farooq, Shagufta Shahbaz, Mohammad Aziz Khan and Maria Sadique (2009). Health Risk Assessment of heavy metals for population via consumption of vegetables. World Applied Sciences Journal, 6, (12): 1602 - 1606

[29] Okunola O. J., Alhassan Y., Yebpella G. G., Uzairu A., Tsafe A. I. , Abechi E. and Apene S. E. (2011). Risk assessment of using coated mobile recharge cards in Nigeria. J. of Env. Chem. and Ecotoxicology, 3, (4): 80 - 85.

[30] USEPA (2009). United States Environmental Protection Agency, Risk-based concentration table. Philadelphia: United States Environmental Protection Agency, Washington, D. C.

[31] USEPA (2008). Child-Specific Exposure Factors Handbook, Chapter 10 - Intake of Fish and Shellfish. Washington, D. C: Office of Research and Development.

[32] Portier K., Tolson J. and Roberts S. (2007). Body weight distributions for risk assessment. Risk Analysis, 27, (1): 11- 26.

[33] USEPA (2011). Exposure Factors Handbook: 2011 Edition. EPA/600/R-090/052F. Office of Research and Development, Washington, D.C.

[34] Standard Operating Procedure (SOP) A03-005. (2008). Method Validation in Chemical Analysis, Issue 3, Rev.0. Sungai Buloh: National Public Health Laboratory.

[35] USEPA (2011). Data validation standard operating procedure for contract laboratory programmed in inorganic data by AAS. Region 4, SOP No. QAS – SOP – 12: 1 – 35.

[36] Abolfazl Askary Sary and Maryam Mohammadi (2012). Comparison of Mercury and Cadmium Toxicity in Fish species from Marine water. Research Journal of Fisheries and Hydrobiology, 7 (1): 14 - 18.

[37] Yilmaz Fevzi (2009). The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (Anguilla Anguilla, Mugil Cephalus and Oreochromis Niloticus) inhabiting Koycegiz Lake-Mugla (Turkey). Turkish Journal of Science Technology, 4, (1): 1 – 15.

[38] Storelli, M. M., Ceci E., Storlli A. and Marcotrigiano G. O. (2003). Polychlorinated biphenyl, heavy metal and methylmercury residues in hammerhead sharks: contaminant status and assesment. Mar. Poll. Bull. (46): 1035 - 1039.

[39] Simon K. D., Othman M. S., Mazlan A. G. , Rahim S. M. and Bashir F. H. (2013). Heavy metal concentration in fishes from the coastal waters of Kapar and Mersing, Malaysia. Turkish Journal of Fisheries and Aquatic Sciences 13: 375 - 382. http://dx.DOI:10.4194/1303-2712-v13_2_21

[40] Gil F. Olmedo P., Pla A., Hernández A.F., Barbier F. and Ayouni L. (2013). Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environment International, (59): 63 – 72.

[41] Sausan A. Omer, Mai A. Ebobeid, Dalia Fouad, Maha H. Daghestani (2012). Cadmium bioaccumulation and Toxicity in tilapia fish (*Oreochromis Niloticus*). Journal of animal and veterinary advances, 11, (10): 1601 – 1606.

[42] Uzairu A., Harrison G. F. S., Balarabe M. L. and Nnaji J. C. (2009). Concentration levels of trace metals in fish and sediment from Kubanni River, Northern Nigeria. Bull. Chem. Soc. Ethiop. 23, (1): 9 - 17.

[43] Fazli H., Zhang C. I., Hay D. E. and Lee C. W. (2009). Stock assessment and management implications of anchovy Kilka in Iranian waters of the Caspian Sea. Fish res. 100: 103 – 108.

[44] Storelli M. M., Normanno G., Barone G., Dambrosio A., Errico L. and Garofalo R. (2012). Toxic metals (Hg, Cd, and Pb) in fishery products imported into Italy: suitability for human consumption. J Food Prot;75.(1):189 – 94.

[45] Copat Chiara , Giovani Arena, Maria Fiore, Caterina Ledda, Roberto Fallico, Salvatore Sciacca and Margherita Ferrante (2013). Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories. Food and Chemical Toxicology, 53: 33 – 37. http://dx.doi.org/10.1016/j.fct.2012.11.038

[46] Kalay M., Ay O. and Canli M. (1999). Heavy metal concentrations in fish tissues from the northeast Mediterranean Sea. Bulletin of Environmental Contamination and Toxicology, 63, (5): 673 – 68

[47] Yazkan M. O. Zedemir F., Golukcu M. (2002). Cu, Zn, Pb and Cd content in some fish species caught in the Gulf of Antalya. Turk J. Vet Anim Sci. 26, (6): 1309 – 13.

[48] CCFAC (Codex Committee on Contaminants in Foods) (2011). Food Standards Programme: Joint FAO/WHO. Fifth Session, The Hague, the Netherlands; 21–25th March.

[49] WHO (2005). Background document for development of WHO Guidelines for Nickel in Drinking-water Quality and Fish. WHO/SDE/WSH/05.08/55 English only.

[50] Joana Raimundo, Patrícia Pereira, Miguel Caetano, Maria Teresa Cabrita and Carlos Vale (2011). Decrease of Zn, Cd and Pb concentrations in marine fish species over a decade as response to reduction of anthropogenic inputs: The example of Tagus estuary. Marine Pollution Bulletin (62): 2854 – 2858. http://dx.doi:10.1016/j.marpolbul.2011.09.020

[51] Usero J. Izquierdo C. Morillo J. and Gracia I. (2004). Heavy metals in fish (Solea vulgaris, Anguilla Anguilla and Liza aurata) from salt marshes on the southern Atlantic coast of Spain. Environ Int. 29, (7): 949 – 56.

[52] Fredrick Asante, Etornyo Agbeko, Georgina Addae and Albert K. Quainoo (2014). Bioaccumulation of heavy metals in water, sediments and tissues of some selected fish from the Red Volta, Nangodi in the upper east region of Ghana. British, Journal of Applied Science & Technology, 4, (4): 594 - 603.

[53] Hulya Karadedea, Seyit Ahmet Oymakb and Erhan Unlu (2004). Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, from the Ataturk Dam Lake (Euphrates), Turkey. Environment International, 30: 183 – 188. http://dx.doi:10.1016/S0160-4120(03): 169 - 176.

[54] Reena Singh, Neetu Gautam, Anurag Mishira and Rajif Gufta (2011). Heavy metals and living system: An over view. Indian J. of Pharmacology, 43, (3): 246 - 253. http://dx.doi.org/10.4103%2F0253-7613.81505

[55] Abdullah El-Ghasham, Mehanna E. E. and Meki Abdel-Reheem (2008). Evaluation of lead and cadmium levels in fresh water fish farms at Qassim region, K.S.A. Journal of Agricultural and Veterinary Sciences, Qassim University, 1, (2): 59 - 77.

[56] Daniel N. Weber and Wendy M. Dinge (1997). Alterations in Neurobehavioral responses in fishes exposed to lead and lead-chelating Agents. Amer. Zool., 37: 354 - 362.

[57] Mieiro C. L., Pacheco M., Pereira M. E., Duarte A. C. (2009). Mercury distribution in key tissues of fish (Liza Aurata) inhabiting a contaminated estuary-implications for human and ecosystem health risk assessment. J. Environ Monit., 11, (5): 1004 - 12. http://dx.doi:10.1039/b821253h

[58] Marioara Nicula, P. Negrea, I. Gergen, M. Harmanescu, I. Gogoasa and M. Lunca (2012). Mercury bioaccumulation in tissues of fresh water fish *carassius auratus gibelio* (Silver Crucian Carp) after chronic mercury intoxication. University de-Ştiinţe Agric and Medicinal Veterinary Iasi Lucrari Ştiinţifice. (52): 676 - 679.

[59] Storelli M. M., Normanno G., Barone G., Dambrosio A., Errico L. and Garofalo R. (2012). Toxic metals (Hg, Cd, and Pb) in fishery products imported into Italy: suitability for human consumption. J Food Prot;75, (1): 189 – 94. http://dx.doi:10.4315/0362-028X.JFP-11-212

[60] Durali Mendil, Zafer Demirci, Mustafa Tuzen and Mustafa Soylak (2010). Seasonal investigation of trace element contents in commercially valuable fish species from the Black sea, Turkey. Food and Chemical Toxicology (48): 865 – 870

[61] Jabeen G., Javed M.and Azmat H. (2012). Assessment of heavy metals in the fish collected from the river Ravi, Pakistan. Pak. Vet. J., 32, (1): 107 - 111.

[62] Zubair Ahmad, El Amin Mohamed Suliman, Khalid Abdullah Al-Ghanim and Shahid Mahboob (2013). Effects of sub-lethal exposure of lead acetate on histopathology of gills, liver, kidney and muscle and its accumulation in these organs of Clarias gariepinus. Braz. arch. biol. technol., 56, (2): 45 – 56. http://dx.doi.org/10.1590/S1516-89132013000200015

[63] Jayashree S. Menon and Sarita Mahajan (2013). Mercury accumulation in different tissues of fish from Ulhas River Estuary and Thane Creek and the pattern of fish consumption among fish eaters. Indian j. Geo-marine scie. 42, (6): 812 - 816.

[64] Lillian Herger and Lorraine Edmond (2012). Assessment of Mercury in Fish Tissue from Select Lakes of Northeastern Oregon, U.S. Environmental Protection Agency Region 10, EPA-910-R-12-001.

[65] Hjeltnes Brit and Kiire Julshamn (1992). Concentrations of iron, copper, zinc and selenium in liver of Atlantic salmon *Salmo salar* infected with *Vibrio salmonicida*. Dis. aquat. 12): 147 – 149.

[66] Sahar Mohammadnabizadeh, Alireza Pourkhabbaz and Reza Afshari (2014). Analysis and determination of trace metals (nickel, cadmium, chromium, and lead) in tissues of *Pampus argenteus* and *Platycephalus indicus* in the Hara Reserve, Iran. Journal of Toxicology, 576496 - 6. http://dx.doi.org/10.1155/2014/576496

[67] Denise Grotto, Bruno Lemos Batista, Maria Fernanda Hornos Carneiro, and Fernando Barbosa Jr. (2012). Evaluation by ICP-MS of Essential, Nonessential and Toxic Elements in Brazilian Fish and Seafood Samples. Food and Nutrition Sciences, 3, 1252 – 1260. http://dx.doi.org/10.4236/fns.2012.39165

[68] Israa A. J., Luma H. Z. and Rana M. A. (2013). Bacterial and heavy metals analyses in fish at Shawaka area of Tigris River. Chemistry and Materials Research, 3, (7): 94 – 100.

[69] Han B. C., Jeng W. L., Chen R. Y., Fang G. T., Hung T. C. and Tseng R. J. (1998). Estimation of Target Hazard Quotients and Potential Health Risks for metals by consumption of Seafood in Taiwan. Arch. Environ. Contam. Toxicol. 35, (1): 711 – 720.

[70] Capelli, R. Das K., Pellegrini R. D., Drava G., Lepoint G. Miglio C. Minganti V. and Poggi R. (2008). Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios. Sci. Total Environ. 390(2-3): 569-578. dx.doi.org/10.1016/j.scitotenv.2007.10.036

[71] Kotze P., Preez H. H., Van J. and Vuren H. J. (1999). Bioaccumulation of copper and zinc in Oreochromis mossambicus and Clarias gariepinus, from the Olifants River, Mpumalanga, South Africa. Water SA. 25, 99 - 110.

[72] Gaspic Z. K., Zvonaric T, Vrgoc N., Odzak N. and Baric A. (2002). Cadmium and lead in selected tissues of two commercially important fish species from the Adriatic Sea. Water Res, (36): 5023 – 5028.

[73] Tom K. Grimsrud, Steinar R. Berge, Tor Haldorsen and Aage Andersen (2002). Exposure to different forms of nickel and risk of lung cancer. Am. J. Epidemiol., 156, (12): 1123 - 1132. http://dx.doi:10.1093/aje/kwf165

APPENDIX

Table 8. Correlation of cadmium concentration (mgL^{-1}) in tissues of *Trachurus Tracurus* from RS fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.872**	1						
Gills	0.360	0.523*	1					
Liver	0.217	0.182	-0.621**	1				
Intestine	0.702**	0.730**	0.426	0.214	1			
Kidney	0.594**	0.628**	0.438	0.205	0.382	1		
Brain	-0.131	0.193	0.204	0.301	0.321	0.266	1	
Bones	-0.277	-0.106	-0.151	0.272	0.100	-0.274	0.607**	1

** Correlation is significant at the 0.01 level (2-tailed) * Correlation is significant at the 0.05 level (2-tailed)

Table 9. Correlation of cadmium concentration (mgL^{-1}) in tissues of *TrachurusTrachurus* from EU fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.642**	1						
Gills	0.174	0.148	1					
Liver	0.489*	0.636**	-0.083	1				
Intestine	0.345	0.409	0.448	0.345	1			
Kidney	-0.419	-0.120	-0.172	0.223	0.077	1		
Brain	-0.277	-0.290	0.051	0.099	0.054	0.139	1	
Bones	0.524*	0.610**	-0.052	0.962**	0.311	0.027	0.232	1

** Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 10 Correlation of lead concentration (mgL^{-1}) in tissues of *Trachurus Trachurus* from RS fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.879**	1						
Gills	0.876**	0.968**	1					
Liver	0.953**	0.938**	0.953**	1				
Intestine	0.861**	0.952**	0.973**	0.942**	1			
Kidney	0.899**	0.953**	0.984**	0.985**	0.971**	1		
Brain	0.936**	0.910**	0.941**	0.984**	0.961**	0.975**	1	
Bones	0.948**	0.972**	0.952**	0.984**	0.927**	0.968**	0.948**	1

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 11. Correlation of lead concentration (mgL^{-1}) in tissues of *Trachurus Trachurus* from EU fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.213	1						
Gills	0.604**	0.657**	1					
Liver	0.347	0.788**	0.865**	1				
Intestine	0.044	0.950**	0.448	0.667**	1			
Kidney	0.229	0.659**	0.420	0.469*	0.524*	1		
Brain	-0.213	0.693**	0.117	0.361	0.695**	0.854**	1	
Bones	-0.456	0.670**	0.046	0.392	0.737**	0.643**	0.933**	1

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 12. Correlation of mercury concentration (mgL^{-1}) in tissues of *Trachurus Trachurus* from RS fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.983**	1						
Gills	0.990**	0.994**	1					
Liver	0.977**	0.991**	0.996**	1				
Intestine	0.976**	0.991**	0.996**	0.997**	1			
Kidney	0.788**	0.819**	0.821**	0.858**	0.823**	1		
Brain	0.994**	0.982**	0.991**	0.978**	0.978**	0.770**	1	
Bones	0.995**	0.990**	0.995**	0.983**	0.986**	0.770**	0.997**	1

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 13. Correlation of mercury concentration (mgL^{-1}) in tissues of *Trachurus Trachurus* from EU fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.922**	1						
Gills	0.979**	0.905**	1					
Liver	0.878**	0.860**	0.927**	1				
Intestine	0.920**	0.878**	0.951**	0.879**	1			
Kidney	0.398	0.624**	0.454	0.442	0.368	1		
Brain	0.380	0.537*	0.513*	0.530*	0.489*	0.890**	1	
Bones	0.604**	0.708**	0.723**	0.705**	0.741**	0.795**	0.945**	1

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 14. Correlation of iron concentration (mgL^{-1}) in tissues of *Trachurus Trachurus* from RS fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.198	1						
Gills	0.184	-0.169	1					
Liver	0.234	0.789**	0.378	1				
Intestine	-0.279	0.818**	0.073	0.817**	1			
Kidney	0.356	0.346	0.414	0.727**	0.329	1		
Brain	0.761**	0.321	0.679**	0.560*	0.114	0.463	1	
Bones	0.827**	0.547*	-0.046	0.335	0.067	0.054	0.673**	1

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 15. Correlation of iron concentration (mgL^{-1}) in tissues of *Trachurus Trachurus* from EU fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.258	1						
Gills	-0.369	-0.236	1					
Liver	0.842**	0.442	-0.021	1				
Intestine	0.863**	0.016	-0.163	0.849**	1			
Kidney	0.381	0.546*	-0.102	0.703**	0.421	1		
Brain	0.241	-0.241	-0.051	0.101	0.035	0.229	1	
Bones	0.756**	-0.220	-0.288	0.424	0.734**	-0.252	-0.044	1

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 16. Correlation of nickel concentration (mgL^{-1}) in tissues of *Trachurus Trachurus* from RS fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.915**	1						
Gills	0.960**	0.903**	1					
Liver	0.734**	0.874**	0.840**	1				
Intestine	0.610**	0.648**	0.726**	0.841**	1			
Kidney	0.823**	0.947**	0.885**	0.961**	0.788**	1		
Brain	0.717**	0.872**	0.808**	0.969**	0.783**	0.923**	1	
Bones	0.782**	0.931**	0.843**	0.953**	0.706**	0.942**	0.976**	1

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 17. Correlation of nickel concentration (mgL^{-1}) in tissues of *Trachurus Trachurus* from EU fishing area

Parameter	Skin	Muscle	Gills	Liver	Intestine	Kidney	Brain	Bones
Skin	1							
Muscle	0.951**	1						
Gills	0.010	0.024	1					
Liver	0.048	0.104	0.978**	1				
Intestine	0.176	0.215	0.966**	0.962**	1			
Kidney	-0.003	0.049	0.946**	0.937**	0.936**	1		
Brain	0.004	0.044	0.993**	0.980**	0.978**	0.960**	1	
Bones	0.075	0.163	0.957**	0.974**	0.981**	0.942**	0.978**	1

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed